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Morra, L ; Moch, H

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# Periostin expression and epithelial-mesenchymal transition in cancer: a review and an update

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**Abstract** Periostin, also called osteoblast-specific factor 2, is a secreted cell adhesion protein, which shares a homology with the insect cell adhesion molecule fasciclin I. It has been shown to be an important regulator of bone and tooth formation and maintenance, and of cardiac development and healing. Recent studies revealed that periostin plays an important role in tumor development and is upregulated in a wide variety of cancers such as colon, pancreatic, ovarian, breast, head and neck, thyroid, and gastric cancer as well as in neuroblastoma. Periostin binding to the integrins activates the Akt/PKB- and FAK-mediated signaling pathways which lead to increased cell survival, angiogenesis, invasion, metastasis, and importantly, epithelial-mesenchymal transition of carcinoma cells. In this review we summarize recent clinicopathological studies that have investigated periostin expression in lung, kidney, prostate, liver cancer, and malignant pleural mesothelioma and discuss the role of periostin isoforms in tumorigenesis and their potential as targets for stroma-targeted anticancer therapy.

**Keywords** Periostin · EMT · Isoform · Stroma · Target

## Background

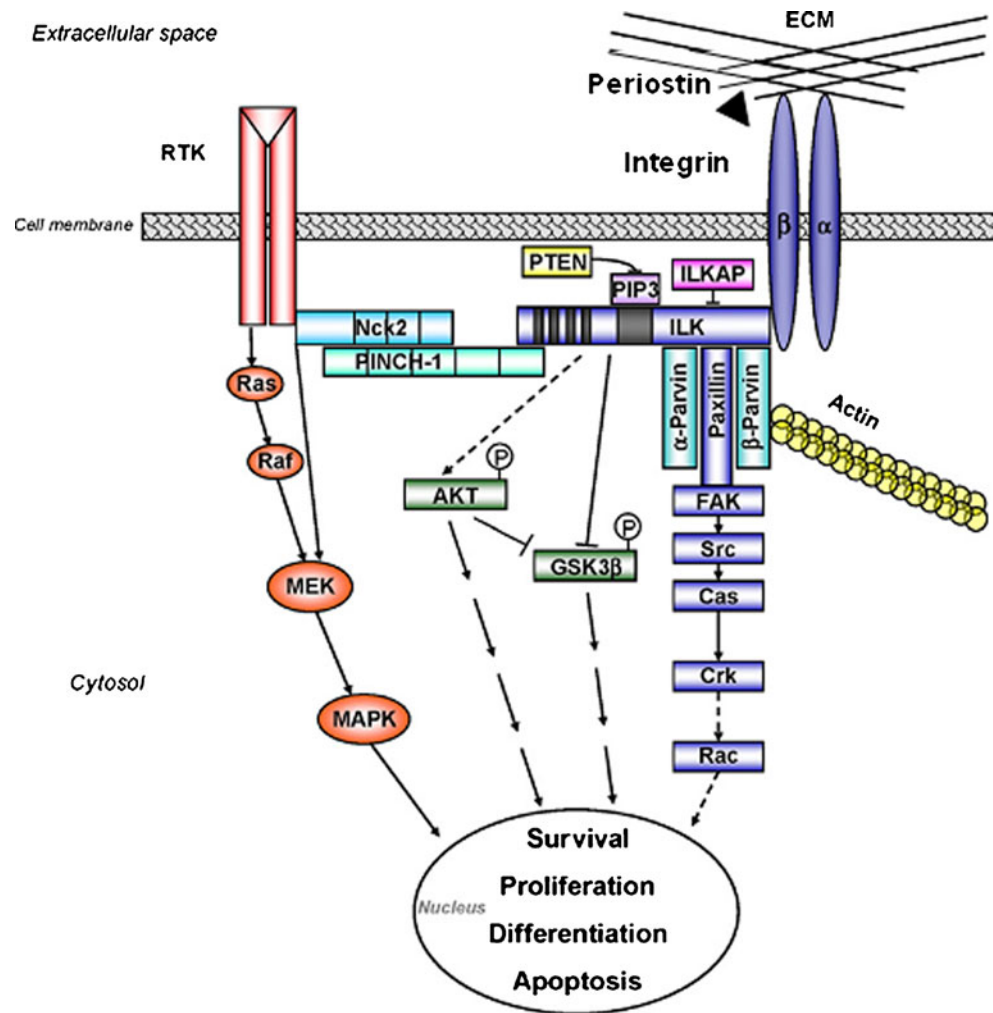
Periostin, also termed osteoblast-specific factor 2, is a 93.3-kDa secreted protein and shares a homology with the insect cell adhesion molecule fasciclin I. It promotes integrin-

dependent cell adhesion and motility and belongs to the superfamily of TGF- $\beta$ -inducible proteins [1, 2]. Its N-terminal region contains a signal peptide (SP) for its secretion, and a cysteine-rich region (EMI domain) which promotes the formation of multimers in non-reducing conditions. Adjacent to the SP and the EMI domains, four internal homologous repeats (FAS domains) are located; these are homologous to the insect cell adhesion protein fasciclin I and act as ligands for the integrins [1, 2]. The C-terminal region of periostin consists of a hydrophilic domain. The N-terminal region of periostin is highly conserved, while the C-terminal region of the protein varies depending on the isoform [1, 3]. The N-terminal region regulates the cell function by binding to integrins at the plasma membrane of the cells through its FAS domains. The C-terminal region of the protein regulates the cell-matrix organization and interactions by binding extracellular matrix (ECM) proteins such as collagen I/V, fibronectin, tenascin C, acid mucopolysaccharides, such as heparin and periostin itself [4]. Periostin was shown to be able to bind the integrins  $\alpha v\beta 3$ ,  $\alpha v\beta 5$ , and  $\alpha 6\beta 4$ , promoting the recruitment of the epidermal growth factor receptor (EGFR) and the activation of the Akt/PKB and FAK-mediated signaling pathways. Periostin-activated signaling pathways promote cellular survival, angiogenesis, and resistance to hypoxia-induced cell death. Additionally, periostin can be upregulated in response to the stress of hypoxia in the human A549 non-small cell lung cancer cell line and in rat pulmonary arterial smooth muscle cells (PASMCs) [5, 6]. The study of Li et al. demonstrated that upregulation of periostin in PASMCs is mediated through Ras signaling [6] (Fig. 1).

The desmoplastic tumor stroma constitutes the so-called tumor microenvironment, which supports growth, invasion, and immune evasion of the tumor. It is formed by a stromal matrix in which cancer cells and the peri-tumoral stromal

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**Fig. 1** Periostin-activated signaling pathways. Periostin binding to integrins initiates the crosstalk between those integrins and the receptor tyrosine kinases (RTKs), such as EGFR, at the plasma membrane. This crosstalk activates the Akt/PKB and the FAK-mediated signaling pathways. Ras signaling activates periostin under hypoxic conditions. Reprinted from [69]



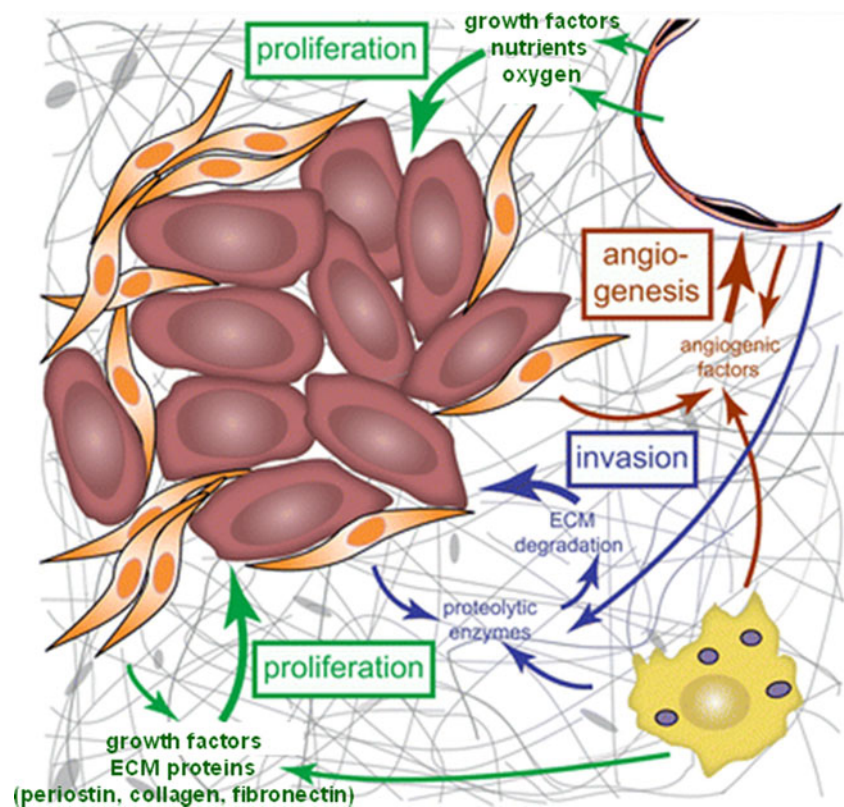
cells are embedded. The tumor stromal cells consist of tumor-associated endothelial cells (TECs), cancer-associated fibroblasts (CAFs), and tumor-associated macrophages, as well as distinct sets of tumor-directed lymphocytes. All these cell types produce growth factors, angiogenic factors, and proteolytic enzymes which sustain the tumor growth and angiogenesis and degrade the ECM, enhancing the tumor cell invasion and metastasis [7]. Furthermore, an excessive production of ECM proteins such as periostin, collagen, and fibronectin may contribute to the generation of a tumor-supportive microenvironment (Fig. 2). Within this context, the matrix protein periostin (gene *POSTN*) plays an important role, regulating cell function and cell–matrix interactions.

Periostin probably exerts its pro-tumorigenic effect not only through its binding to the integrins and the consequent activation of intracellular pathways which determine an enhanced invasiveness, but also through its effect on the ECM fibrillogenesis. The C-terminal region of periostin has been shown to interact with ECM molecules by immunoprecipitation and binding

assays [4, 8]. Together with those studies showing colocalization of periostin with fibronectin, collagen, and tenascin C [8], these findings suggest that periostin regulates collagen fibrillogenesis and biomechanical properties of connective tissues, forming reticular structures. Considering that alterations in the ECM components of the tumor microenvironment have a remarkable impact on the invasive and metastatic process, it is possible that periostin promotes an ECM organization that supports invasion and metastasis (Fig. 3).

Periostin protein is physiologically expressed in a wide variety of normal adult tissues and fetal tissues, such as embryonic periosteum, periodontal ligament, placenta, cardiac valves, adrenal glands, lung, thyroid, stomach, colon, vagina, ovary, testis, prostate, and breast [1, 9]. Moreover, periostin is expressed in tissues under stress conditions, such as heart under pressure or volume overload, skeletal muscle after injury, and in pulmonary aortic smooth muscle cells in response to hypoxia [6, 10, 11]. Periostin has been shown to be essential for bone and tooth formation and maintenance [2], for heart develop-

**Fig. 2** Tumor microenvironment and its influence on tumor development. Tumor stromal cells, e.g., CAFs, TECs, and tumor-associated macrophages (TAMs), express growth factors sustaining tumor growth. Among these are angiogenic factors promoting angiogenesis, proteolytic enzymes catalyzing the degradation of the ECM, and ECM proteins creating a tumor supporting environment. All these factors together regulate tumor cell growth, invasion, and finally metastasis. Tumor cells are depicted in brown, CAFs in orange, TECs in red, and TAMs in yellow. Reprinted from [7]



ment, and healing after acute myocardial infarction [12]. Periostin-deficient mice have a mild phenotype characterized by alterations in periodontal ligament and craniofacial ECM [13], structural valvular anomalies [14], and reduced fibrosis after myocardial infarction [15].

Periostin upregulation has been reported for many cancer types, as non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), malignant pleural mesothelioma (MPM), and others [16–21] and is consequently defined as a tumor-enhancing factor. Only a few reports in bladder cancer and osteosarcoma have described periostin as a tumor-inhibiting factor [22, 23]. Moreover periostin upregulation has been reported also for chronic sinusitis and recent studies suggest that periostin may be part of a negative-feedback loop regulating allergic airway inflammation [24, 25]. Importantly, periostin is involved in the epithelial-mesenchymal transition (EMT) of carcinoma cells [6, 17, 19, 26, 27].

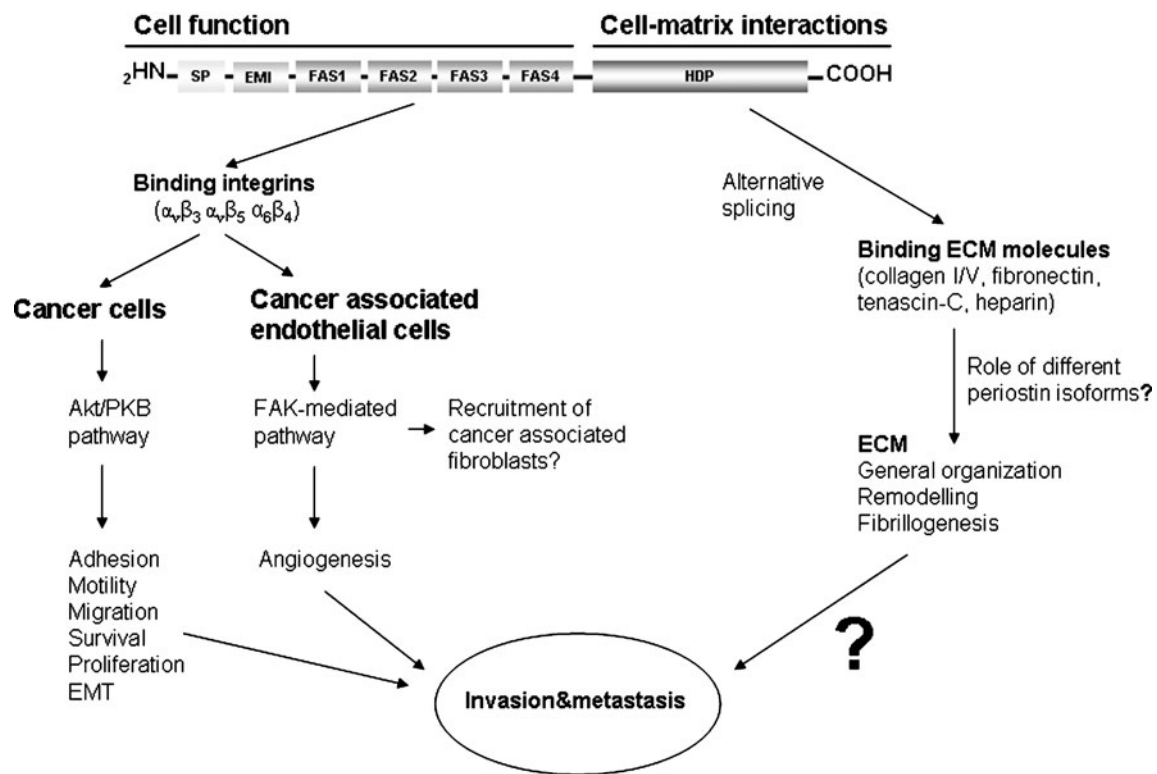
In this review, we summarize the current knowledge about the role of periostin in the process of EMT and discuss its potential as a target for anticancer therapies directed against the tumor stroma. Furthermore, we summarize current knowledge about periostin expression in the different tumor components of NSCLC, MPM, prostate cancer (PC), RCC, hepatocellular carcinoma (HCC), and bile duct carcinoma (BDC) [16, 20, 21, 28, 29] (Table 1).

### Epithelial-mesenchymal transition and periostin

It is well known that the EMT process is fundamental during the embryonic development of multicellular organisms [30]. In addition to embryonic development, the EMT also participates in tumor progression. This is not unexpected, considering that analogies between the two processes (embryonic development and tumor progression) frequently occur. An increasing number of genes and signaling pathways that are involved in embryonic development are also found to be involved in tumorigenesis [31]. However there is much evidence that the EMT process is responsible for dissemination of primary tumor epithelial cells to the sites of metastasis and for the dedifferentiation program that leads to increased malignant behavior of the tumor [30] (Fig. 4).

On a morphologic basis, EMT *in vitro* can be recognized by the presence of spindle-shaped cells, emission of pseudopodia, and increased intercellular separation, which reflects the acquisition of a mesenchymal phenotype, increased motility, and loss of cell-cell adhesion. Furthermore, the EMT of cancer cells is characterized by an upregulation of mesenchymal proteins such as periostin, vimentin, fibronectin, and N-cadherin, accompanied by a downregulation of epithelial markers such as E-cadherin.

## Periostin



**Fig. 3** The role of periostin in cancer. The N-terminal region of the protein influences cell function, while the C-terminal influences cell-matrix interactions. Through its FAS domains, periostin binds to integrins and activates the Akt/PKB and the FAK-mediated signaling pathways, leading to increased tumor invasion and metastasis. The C-

terminal region of periostin binds extracellular matrix (ECM) molecules and influences the general organization of the extracellular matrix. Periostin splice isoforms differ in the C-terminal and it is still not known if they contribute differentially to ECM formation and to the global invasive and metastatic potential of the protein

On a functional basis, gain of N-cadherin expression, together with loss of E-cadherin, leads to alterations in the epithelial cell shape and motility by dissociation of intercellular adherens junctions, promoting only weak homophilic cell adhesion interactions [30, 31]. In vitro, lack of E-cadherin determines acquisition of a mesenchymal phenotype and invasive behavior, which can be

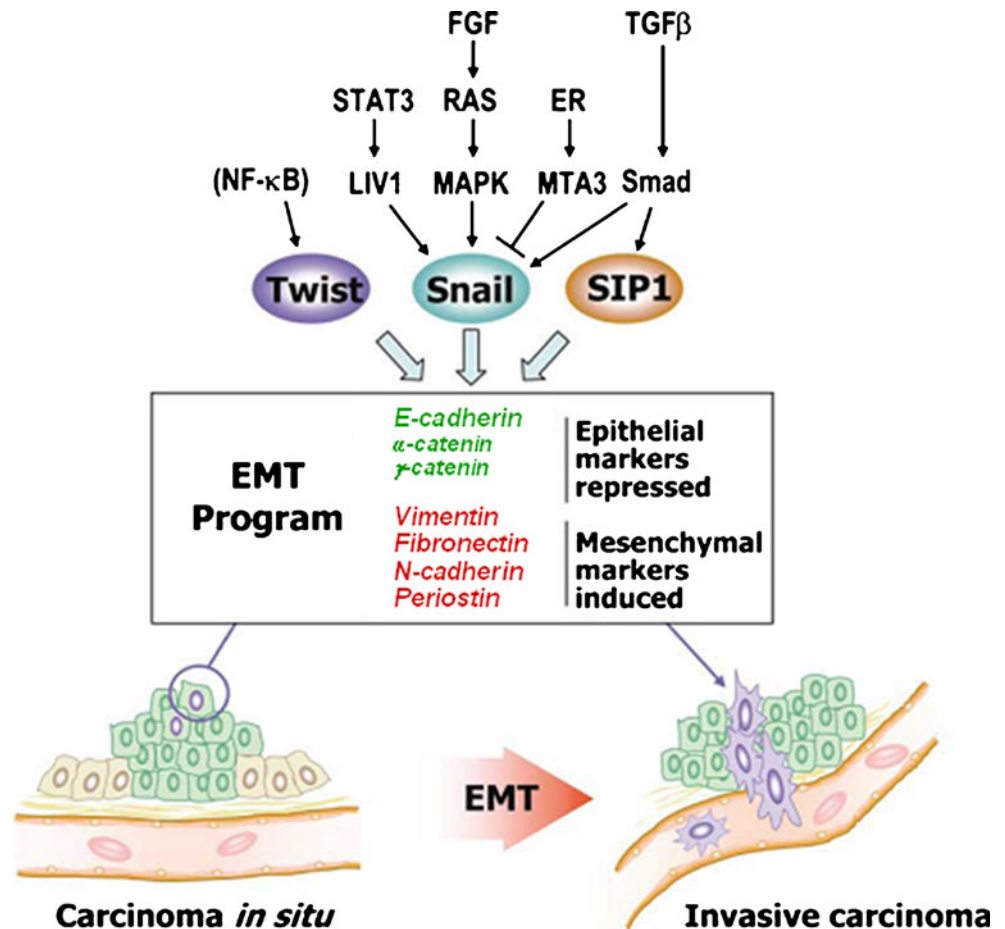
reversed if E-cadherin is restored and constitutively expressed [32]. In vivo, it has been shown that E-cadherin-negative cell lines have an increased tumorigenic potential when implanted as xenografts in nude mice [30]. Clinical studies showed that E-cadherin expression is inversely correlated with high-grade cancers or with poor survival [33, 34]. On a molecular basis, E-cadherin is

**Table 1** Overview of correlations found, by immunohistochemical studies on NSCLC, MPM, PC, RCC, and BDC tissue microarrays (TMAs), between epithelial and stromal periostin expression and clinicopathological parameters

Tumor type	Poor survival	Differentiation grade	Stage of disease	Reference
Non-small cell lung cancer	Stromal expression		Epithelial and stromal expression	Soltermann et al. [16]
Malignant pleural mesothelioma	Epithelial expression			Schramm et al. [20]
Prostate cancer	Stromal expression	Epithelial and stromal expression	Epithelial expression	Tischler et al. [28]
Renal cell carcinoma	Epithelial expression	Epithelial and stromal expression	Epithelial expression	Dahinden et al. [21]
Bile duct carcinoma	Epithelial expression			Riener et al. [29]



**Fig. 4** Drivers and mediators of EMT. Early stage tumor cells (green) maintain epithelial properties similar to the neighboring normal epithelium (brown). The overexpression of master regulators of EMT, such as *Twist*, *Snail*, and *SIP1* in cancer cells (shown with purple nuclei) leads to dramatic changes in the gene expression profile and cellular behavior. *Twist*, *Snail*, and *SIP1* suppress the expression of E-cadherin and trigger expression of an entire EMT transcriptional program through as yet unknown mechanisms. Reprinted from [31]



downregulated by epigenetic mechanisms, such as promoter hypermethylation and transcriptional repression. Transcriptional repressors of E-cadherin are the zinc finger proteins Snail and SIP1 and the basic helix-loop-helix transcription factor Twist, all of which have the ability to recognize and bind E boxes in the E-cadherin promoter [35–37]. Snail, SIP1, and Twist are therefore inducers of EMT and promote tumor cell invasion. Activation of several growth factor receptors, including tyrosine kinase receptors such as the hepatocyte growth factor receptor c-Met, the ErbB protein family, and the fibroblast growth factor, the insulin growth factor, and the transforming growth factor- $\beta$  (TGF- $\beta$ ) receptors, have been found to induce EMT in vitro and in vivo [38–41]. Downstream effectors of these activated receptors belong to the Ras and the mitogen-activated protein kinase pathway, the phosphoinositide 3-kinase pathway, or to the TGF- $\beta$ -SMAD pathway, which all lead to Snail expression [30, 31, 35]. Recently, the DNA binding factor LIV1 has been found to promote activation of Snail through a STAT3-dependent pathway [42] (Fig. 4).

The matrix protein periostin was shown to be not only a marker of EMT, but to be itself an inducer of this phenomenon [26, 27]. Indeed Yan et al. demonstrated that

ectopic expression of periostin in tumorigenic but non-metastatic 293T cells can induce EMT and promote invasion and metastasis in vivo [26]. Upregulation of periostin was accompanied by upregulation of vimentin, fibronectin, and active MMP-9, while E-cadherin and N-cadherin expression was unaltered. Periostin signaling pathway in 293T cells seems to require interaction with  $\alpha\beta 5$  integrin and recruitment of EGFR, as demonstrated by the fact that periostin-induced increase in cell adhesion, migration and invasion can be blocked by incubation with anti-integrin  $\alpha\beta 5$  antibody or with tyrphostin 25, an EGFR kinase inhibitor.

Kim et al. found that periostin was able to induce EMT and an increased invasiveness in prostate cancer cell lines by downregulation of E-cadherin via Snail and increased phosphorylation of Akt [27]. Opposite effects were observed using bladder cancer cell lines, indicating that periostin regulates E-cadherin in a cell-type-dependent way. Furthermore, the C-terminal region of periostin was not sufficient for induction of EMT in prostate cancer cells, indicating that the induction of EMT is mediated by the N-terminal region of periostin.

However, examination of the expression pattern of EMT markers (such as periostin, vimentin, fibronectin, N-

cadherin, E-cadherin, and  $\beta$ -catenin) or members of the Akt/PKB signaling pathway allows determination of their occurrence and clinical significance in vivo. Therefore, an increasing number of immunohistochemical studies aim to characterize EMT expression profiles and their prognostic significance.

### Periostin expression in different tumor types

Upregulation of periostin has been observed in many cancer types, such as neuroblastoma [43], head and neck [44], nasopharyngeal [45], thyroid [46], oral [47], breast [48], and ovarian cancer [19]. Elevated periostin levels were also detected in the serum from thymoma [49] and breast [50] cancer patients as well as in serum and pleural effusions of lung cancer patients [51, 52]. In the following, recent data on periostin expression will be described for different tumor types.

### Non-small cell lung cancer

Periostin expression in normal healthy lung tissue has been observed [9] and its upregulation in cancerous lung tissue has been reported [16, 53] and associated with EMT [16]. Recently, periostin was identified in malignant pleural effusions from lung adenocarcinoma by mass spectrometric N-glycoprotein profiling [52] and its serum levels were shown to be higher compared with the normal control in NSCLC. The normal control consisted of pleural effusions from patients with cardiac disease but no neoplasia. Soltermann et al. compared the expression of EMT proteins such as periostin and vimentin, the MET protein versican, and classical desmoplasia markers such as collagen and elastin, in NSCLC by immunohistochemical analysis of tissue microarrays (TMAs). From these studies, it was derived that stromal periostin is a prognostic factor for decreased progression-free survival in NSCLC, and together with epithelial periostin, it was also significantly associated with several clinicopathological parameters such as squamous cell carcinoma histotype, higher stage, higher pT, higher pM, larger tumor size, and vessel infiltration, as well as with male gender [16]. Moreover, stromal periostin expression was significantly associated with epithelial cytoplasmic expression of vimentin and with collagen, but not with elastin, suggesting that during tumor progression the stromal fibrillogenesis in NSCLC may switch from elastin to periostin [16]. However, periostin stromal expression in NSCLC is mostly due to the intra-tumoral component, rather than to the peritumoral component of the stroma (own unpublished data). Interestingly, periostin was also correlated with the MET marker versican [16]; the expression of versican has been

shown to be associated with tumor recurrence and more advanced disease in NSCLC [54].

### Malignant pleural mesothelioma

MPM consists of different histotypes, including epithelioid, sarcomatoid, and biphasic. The biphasic MPM is a mixed histotype which is associated with both epithelial and mesenchymal phenotypes. The sarcomatoid and epithelioid histotypes resemble the EMT–MET transdifferentiation, being associated with the epithelial and mesenchymal differentiation state of the cell, respectively.

Schramm et al. conducted an immunohistochemical study on more than 350 MPM patients. Members of a putative MPM–EMT signaling cascade were analyzed: the EMT marker periostin, the EGFR, integrin  $\beta$ 1, the inhibitor of Akt signaling pathway phosphatase and tensin homolog (PTEN), the integrin-linked kinase (ILK), and the two cell cycle regulators, p21 and p27. The results showed that expression of periostin in tumor cells can be considered as an independent prognostic factor for overall survival [20]. Indeed high periostin levels in tumor cells correlated with poor survival, whereas high cytoplasmic PTEN, ILK, or nuclear p21 and p27 correlated with better survival.

Interestingly, both stromal and tumor cell periostin expression was associated with the sarcomatoid histotype, indicating that periostin is associated with the mesenchymal differentiation state of the cell [20]. Contrary to periostin, high EGFR and integrin  $\beta$ 1 expression nuclear p27 were associated with the epithelioid histotype of MPM.

### Prostate cancer

A limited number of studies have investigated periostin expression in prostate cancer [9, 28, 55]. Periostin expression in normal prostate tissue has been detected by western blot analysis [9]. Tsunoda et al. detected periostin among several genes by a screening for three-dimensional culture (3DC)-associated genes. Non-malignant prostatic epithelial cells, cultured in two-dimensional culture (2DC) and 3DC, were compared with 40 PC samples and their matched non-neoplastic prostate epithelium. Candidate genes were further validated by quantitative real time (RT)-PCR. Since periostin mRNA showed the highest levels among all the genes upregulated in 3DC, its protein expression was further evaluated by immunohistochemistry (IHC). Cancer cells of early stage but not advanced stage prostate cancer had an increased periostin expression compared with normal glands [55]. Tischler et al. [28] analyzed periostin expression by IHC in more than 400 prostate tumors. Contrary to Tsunoda et al., both stromal

and epithelial periostin expression was found to be increased in advanced and metastatic tumors [28]. They also reported increased periostin expression in the tumor stroma compared with the stroma around normal prostate glands. Furthermore, they showed that stromal periostin is upregulated in high-grade prostate cancers and affirmed stromal periostin as a progression factor for prostate-specific antigen relapse-free survival.

### Renal cell carcinoma

Periostin was identified as an accessible biomarker from the blood stream after ex vivo perfusion and biotinylation of surgically resected human kidney carcinoma [56]. Its tumor specificity was further confirmed by IHC. Periostin showed a prominent vascular and stromal staining pattern [56]. Interestingly, the MET protein versican and the EMT protein periostin were found to have similar stromal expression patterns in RCC [56] as well as in NSCLC [16].

A bioinformatic network modeling approach, comparing immunohistochemical stainings from TMAs of RCC patients, analyzed periostin along with several other proteins to elucidate molecular pathways influenced by the von Hippel-Lindau (*VHL*) gene and the *PTEN* gene, which are often deregulated in clear cell RCC [21]. Both epithelial and a stromal periostin staining was observed. In particular, epithelial periostin was significantly increased in high-grade and high-stage tumors and was significantly associated with poor overall survival [21]. In an estimated graphical log-linear model, periostin was also found to be positively associated with nuclear *PTEN*, phosphorylated ribosomal protein p-S6, and nuclear p21, whereas cytoplasmic *PTEN* and inactive non-phosphorylated ribosomal protein S6 correlated with a prolonged survival [21]. Recently, we identified several periostin isoforms in renal tissue by direct sequencing, one of which was found to be tumor associated [57].

### Hepatocellular and bile duct carcinomas

Periostin has been found to enhance tumor progression in several gastrointestinal cancers such as colon, gastric, and pancreatic cancer [17, 58, 59]. Few studies have investigated periostin expression in human liver [18, 29, 60]. Tilman et al. analyzed periostin mRNA levels by quantitative RT-PCR in six HCC and five normal liver samples: periostin mRNA levels were increased by an average 65-fold in tumor [60]. Additionally, among various tumor types, strong periostin upregulation was mostly observed in liver and pancreatic tumors [60]. Baril et al. analyzed periostin expression by IHC in multiple tumor types

including 11 HCC: liver tumors showed the highest periostin levels, together with breast, pancreatic, colon and larynx tumors. Recently, Riener et al. [29] performed the first immunohistochemical analysis on a large number of liver cancer patients (91 HCC and 116 BDC), which represented also the first report on periostin expression in BDC. Their study showed that expression of epithelial periostin was increased in higher-grade HCC and was shown to be an independent prognostic marker for poor overall survival in BDC. Periostin expression was also detected in the stroma of both HCC and BDC but did not correlate with clinicopathological parameters. Periostin expression was also analyzed in normal bile ducts, gallbladder epithelium, and hepatocytes, which showed a weak periostin cytoplasmic expression.

These immunohistochemical studies show that, depending on the cancer type, either epithelial or stromal periostin expression is associated with different tumor behavior (Table 1). One might hypothesize the existence of an autocrine/paracrine periostin loop between stromal and epithelial tumor cells and that the protein is secreted at a site where it can exert its strongest effect, namely at the interface between tumor and stroma. Furthermore, different splice variants of periostin might result in different biological effects, if they are secreted either by tumor stromal cells or by tumor epithelial cells.

### Periostin isoforms and alternative splicing

Up to six different splice isoforms of periostin have been reported, four of which have been fully sequenced and annotated [3, 56] (Table 2). The isoforms of periostin are between 83 and 93 kDa in mass and differ in their C-terminal sequences, characterized by individual presence or absence of cassette exons 17–21 (UniProtKB/Swiss-Prot, March 2011); its N-terminus is conserved. Periostin isoforms were initially detected in pre-osteoblast cells, and it has been shown that the levels of osteoblast-specific differentiation markers were markedly reduced when the activity of these proteins was blocked in the mouse

**Table 2** Schematic representation of the periostin cassette exons in the four known isoforms (isoforms 1–4)

Isoform	Exon						
1	1–16	17	18	19	20	21	22–23
2	1–16			19	20	21	22–23
3	1–16		18	19	20		22–23
4	1–16			19	20		22–23

Periostin splice isoforms are characterized by an individual presence or absence of cassette exons 17–21



osteoblast cell line MC3T3-E1. These results suggest that these proteins play a role in the differentiation of osteoblasts [3].

The periostin C-terminal domain probably modulates the protein function by binding to ECM molecules such as fibronectin and collagen [61]. Indeed, Norris et al. demonstrated that the C-terminal domain of periostin binds collagen I, concluding that it is involved in fibrillogenesis and influences the biomechanical properties of fibrous connective tissues [4]. On the basis of comparative studies of periostin sequences among vertebrates, Hoersch and Andrade-Navarro suggested that the C-terminal domain of periostin is able to bind the matrix proteins collagen and fibronectin, and that each isoform may exert its influence on the ECM fibrillogenesis differently [61]. Therefore, alternative splicing of periostin isoforms seems to reflect the differential modulation of the periostin function in the ECM. This hypothesis is supported by the finding that downregulation and/or loss by alternative splicing of the periostin non-spliced form, together with expression of variant I (isoform 4 in UniProtKB/Swiss-Prot database), promotes increased invasiveness in *in vitro* and *in vivo* experiments using the human bladder cancer cell line SBT991, the mouse malignant melanoma cell line B16F10, and nude mice [22, 62]. Given that periostin isoforms have a differential invasive potential, it remains to be clarified how exactly periostin isoforms modulate the ECM formation and if this periostin-isoform-modulated ECM formation has an influence on the global invasive and metastatic potential of the protein.

Alternative splicing contributes to the expansion of transcriptomic diversity. It is achieved by the interplay of several RNA-binding proteins that associate with pre-mRNA transcripts, determining an exon skipping or inclusion in the mature RNA transcript [63]. Several ubiquitous families of proteins that regulate splicing have been identified and they act in cooperation with tissue-specific regulators of splicing [64]. Warzecha et al. identified the epithelial splicing regulatory proteins 1 and 2 (ESRP1 and ESRP2) as cell-type-specific regulators of transcripts that switch splicing during the EMT [65]. Those two RNA-binding proteins promote alternative splicing events with function in cell–cell adhesion, polarity, and migration, influencing the phenotypic morphological changes that are observed in the EMT. This complex alternative splicing network reveals an important post-transcriptional aspect in addition to the changes in gene expression that underlie the EMT. In particular, it was shown that downregulation of the ESRPs leads to an increase in exon skipping events in the penultimate exons of the C-terminal transcript regions of all analyzed proteins. These events are thought to be functionally relevant because the skipped exons were in multiples of three

nucleotides, allowing translation into different protein isoforms. Even though periostin was not identified among the transcripts regulated by ESRP1 and ESRP2, periostin isoforms are generated by alternative splicing in the penultimate exons of the C-terminal transcript region and the skipped exons are in multiples of three. Therefore it is possible that periostin transcripts are regulated during EMT by specific splice-regulatory proteins and that those transcripts have an influence on the morphologic and functional changes associated with the EMT.

### Periostin as a target for immunotherapy

In RCC, periostin was found to be overexpressed in the subendothelial stroma of the tumor neovasculature [56]. It was suggested that periostin represents a potential target for anticancer therapies directed against the subendothelial stroma (so called “tumor vascular targeting” [66]). Indeed, the tumor neovasculature is tumor specific and discrete from normal adult tissue, except for a local and transient angiogenesis that occurs during specific processes such as tissue regeneration and inflammation [67].

Castronovo et al. found periostin to be an accessible antigen from the blood stream after *ex vivo* perfusion and biotinylation of surgically resected human renal carcinomas [56]. The *ex vivo* biotinylation of surgically resected renal carcinomas allowed a selective labeling of tumor vascular structures. Subsequent proteomic analysis of the biotinylated specimens allowed the identification of several proteins in the tumor portions, including periostin, which was the most abundant tumor-associated protein identified in RCC [56]. Validation of periostin as a tumor-associated antigen was performed by IHC and PCR. IHC showed overexpression of periostin in tumor samples, with a prominent vascular and stromal pattern of staining. PCR analysis yielded stronger periostin expression in fetal and tumor specimens [56]. These data suggested that oncofetal periostin variants might represent even more specific tumor targets.

In conclusion, upregulation in tissues and body fluids of many tumor types, accessibility from the blood stream, and correlation with malignant behavior and with poor prognosis speak in favor of the potential usefulness of periostin as a target for immunotherapy. Further, ECM proteins such as periostin, fibronectin, or tenascin C belong to a class of proteins that can be considered suitable tumor antigens for targeted delivery of therapeutic agents because of their accessibility, specificity, stability, and abundance [56, 66]. The therapeutic potential of periostin has recently been evaluated by Castronovo et al. [56] and by Kudo et al. [68], with the conclusion that stroma-targeting represents a valid option in anticancer therapy.

**Conflict of interests** The authors declare no conflict of interest.

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## References

1. Takeshita S, Kikuno R, Tezuka K, Amann E (1993) Osteoblast-specific factor 2: cloning of a putative bone adhesion protein with homology with the insect protein fasciclin I. *Biochem J* 294(Pt 1):271–278
2. Horiuchi K, Amizuka N, Takeshita S, Takamatsu H, Katsuura M, Ozawa H, Toyama Y, Bonewald LF, Kudo A (1999) Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor beta. *J Bone Miner Res* 14:1239–1249
3. Litvin J, Selim AH, Montgomery MO, Lehmann K, Rico MC, Devlin H, Bednarik DP, Safadi FF (2004) Expression and function of periostin-isoforms in bone. *J Cell Biochem* 92:1044–1061
4. Norris RA, Damon B, Mironov V, Kasyanov V, Ramamurthi A, Moreno-Rodriguez R, Trusk T, Potts JD, Goodwin RL, Davis J, Hoffman S, Wen X, Sugi Y, Kern CB, Mjaatvedt CH, Turner DK, Oka T, Conway SJ, Molkentin JD, Forgacs G, Markwald RR (2007) Periostin regulates collagen fibrillogenesis and the biomechanical properties of connective tissues. *J Cell Biochem* 101:695–711
5. Ouyang G, Liu M, Ruan K, Song G, Mao Y, Bao S (2009) Upregulated expression of periostin by hypoxia in non-small-cell lung cancer cells promotes cell survival via the Akt/PKB pathway. *Cancer Lett* 281:213–219
6. Li P, Oparil S, Feng W, Chen YF (2004) Hypoxia-responsive growth factors upregulate periostin and osteopontin expression via distinct signaling pathways in rat pulmonary arterial smooth muscle cells. *J Appl Physiol* 97:1550–1558, discussion 1549
7. Hofmeister V, Schrama D, Becker JC (2008) Anti-cancer therapies targeting the tumor stroma. *Cancer Immunol Immunother* 57:1–17
8. Takayama G, Arima K, Kanaji T, Toda S, Tanaka H, Shoji S, McKenzie AN, Nagai H, Hotokebuchi T, Izuhara K (2006) Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals. *J Allergy Clin Immunol* 118:98–104
9. Tai IT, Dai M, Chen LB (2005) Periostin induction in tumor cell line explants and inhibition of in vitro cell growth by anti-periostin antibodies. *Carcinogenesis* 26:908–915
10. Katsuragi N, Morishita R, Nakamura N, Ochiai T, Taniyama Y, Hasegawa Y, Kawashima K, Kaneda Y, Ogihara T, Sugimura K (2004) Periostin as a novel factor responsible for ventricular dilation. *Circulation* 110:1806–1813
11. Goetsch SC, Hawke TJ, Gallardo TD, Richardson JA, Garry DJ (2003) Transcriptional profiling and regulation of the extracellular matrix during muscle regeneration. *Physiol Genomics* 14:261–271
12. Conway SJ, Molkentin JD (2008) Periostin as a heterofunctional regulator of cardiac development and disease. *Curr Genomics* 9:548–555
13. Rios H, Koushik SV, Wang H, Wang J, Zhou HM, Lindsley A, Rogers R, Chen Z, Maeda M, Kruzynska-Frejtag A, Feng JQ, Conway SJ (2005) Periostin null mice exhibit dwarfism, incisor enamel defects, and an early-onset periodontal disease-like phenotype. *Mol Cell Biol* 25:11131–11144
14. Snider P, Hinton RB, Moreno-Rodriguez RA, Wang J, Rogers R, Lindsley A, Li F, Ingram DA, Menick D, Field L, Firulli AB, Molkentin JD, Markwald R, Conway SJ (2008) Periostin is required for maturation and extracellular matrix stabilization of noncardiomyocyte lineages of the heart. *Circ Res* 102:752–760
15. Oka T, Xu J, Kaiser RA, Melendez J, Hambleton M, Sargent MA, Lorts A, Brunskill EW, Dorn GW 2nd, Conway SJ, Aronow BJ, Robbins J, Molkentin JD (2007) Genetic manipulation of periostin expression reveals a role in cardiac hypertrophy and ventricular remodeling. *Circ Res* 101:313–321
16. Soltermann A, Tischler V, Arbogast S, Braun J, Probst-Hensch N, Weder W, Moch H, Kristiansen G (2008) Prognostic significance of epithelial-mesenchymal and mesenchymal-epithelial transition protein expression in non-small cell lung cancer. *Clin Cancer Res* 14:7430–7437
17. Bao S, Ouyang G, Bai X, Huang Z, Ma C, Liu M, Shao R, Anderson RM, Rich JN, Wang XF (2004) Periostin potently promotes metastatic growth of colon cancer by augmenting cell survival via the Akt/PKB pathway. *Cancer Cell* 5:329–339
18. Baril P, Gangeswaran R, Mahon PC, Caulee K, Kocher HM, Harada T, Zhu M, Kalthoff H, Crnogorac-Jurcevic T, Lemoine NR (2007) Periostin promotes invasiveness and resistance of pancreatic cancer cells to hypoxia-induced cell death: role of the beta4 integrin and the PI3k pathway. *Oncogene* 26:2082–2094
19. Gillan L, Matei D, Fishman DA, Gerbin CS, Karlan BY, Chang DD (2002) Periostin secreted by epithelial ovarian carcinoma is a ligand for alpha(V)beta(3) and alpha(V)beta(5) integrins and promotes cell motility. *Cancer Res* 62:5358–5364
20. Schramm A, Opitz I, Thies S, Seifert B, Moch H, Weder W, Soltermann A (2010) Prognostic significance of epithelial-mesenchymal transition in malignant pleural mesothelioma. *Eur J Cardiothorac Surg* 37:566–572
21. Dahinden C, Ingold B, Wild P, Boysen G, Luu VD, Montani M, Kristiansen G, Sulser T, Buhlmann P, Moch H, Schraml P (2010) Mining tissue microarray data to uncover combinations of biomarker expression patterns that improve intermediate staging and grading of clear cell renal cell cancer. *Clin Cancer Res* 16:88–98
22. Kim CJ, Yoshioka N, Tambe Y, Kushima R, Okada Y, Inoue H (2005) Periostin is down-regulated in high grade human bladder cancers and suppresses in vitro cell invasiveness and in vivo metastasis of cancer cells. *Int J Cancer* 117:51–58
23. Yoshioka N, Fuji S, Shimakage M, Kodama K, Hakura A, Yutsudo M, Inoue H, Nojima H (2002) Suppression of anchorage-independent growth of human cancer cell lines by the TRIF52/periostin/OSF-2 gene. *Exp Cell Res* 279:91–99
24. Sehra S, Yao W, Nguyen ET, Ahly AN, Tuana FM, Ahlfeld SK, Snider P, Tepper RS, Petrache I, Conway SJ, Kaplan MH (2011) Periostin regulates goblet cell metaplasia in a model of allergic airway inflammation. *J Immunol* 186:4959–4966
25. Platt MP, Soler ZM, Kao SY, Metson R, Stankovic KM (2011) Topographic gene expression in the sinonasal cavity of patients with chronic sinusitis with polyps. *Otolaryngology—head and neck surgery. Am Acad Otolaryngol Head Neck Surg* 145:171–175
26. Yan W, Shao R (2006) Transduction of a mesenchyme-specific gene periostin into 293T cells induces cell invasive activity through epithelial-mesenchymal transformation. *J Biol Chem* 281:19700–19708
27. Kim CJ, Sakamoto K, Tambe Y, Inoue H (2011) Opposite regulation of epithelial-to-mesenchymal transition and cell inva-

- siveness by periostin between prostate and bladder cancer cells. *Int J Oncol* 38:1759–1766
28. Tischler V, Fritzsche FR, Wild PJ, Stephan C, Seifert HH, Riener MO, Hermanns T, Mortezaei A, Gerhardt J, Schraml P, Jung K, Moch H, Soltermann A, Kristiansen G (2010) Periostin is up-regulated in high grade and high stage prostate cancer. *BMC Cancer* 10:273
  29. Riener MO, Fritzsche FR, Soll C, Pestalozzi BC, Probst-Hensch N, Clavien PA, Jochum W, Soltermann A, Moch H, Kristiansen G (2010) Expression of the extracellular matrix protein periostin in liver tumours and bile duct carcinomas. *Histopathology* 56:600–606
  30. Thiery JP (2002) Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer* 2:442–454
  31. Kang Y, Massague J (2004) Epithelial-mesenchymal transitions: twist in development and metastasis. *Cell* 118:277–279
  32. Chen WC, Obrink B (1991) Cell-cell contacts mediated by E-cadherin (uvomorulin) restrict invasive behavior of L-cells. *J Cell Biol* 114:319–327
  33. Oka H, Shiozaki H, Kobayashi K, Inoue M, Tahara H, Kobayashi T, Takatsuka Y, Matsuyoshi N, Hirano S, Takeichi M et al (1993) Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. *Cancer Res* 53:1696–1701
  34. Hirohashi S (1998) Inactivation of the E-cadherin-mediated cell adhesion system in human cancers. *Am J Pathol* 153:333–339
  35. Nieto MA (2002) The snail superfamily of zinc-finger transcription factors. *Nat Rev Mol Cell Biol* 3:155–166
  36. Comijn J, Berx G, Vermassen P, Verschueren K, van Grunsven L, Bruyneel E, Mareel M, Huylebroeck D, van Roy F (2001) The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion. *Mol Cell* 7:1267–1278
  37. Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A, Weinberg RA (2004) Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 117:927–939
  38. Khoury H, Dankort DL, Sadekova S, Naujokas MA, Muller WJ, Park M (2001) Distinct tyrosine autophosphorylation sites mediate induction of epithelial mesenchymal like transition by an activated ErbB-2/Neu receptor. *Oncogene* 20:788–799
  39. Meiners S, Brinkmann V, Naundorf H, Birchmeier W (1998) Role of morphogenetic factors in metastasis of mammary carcinoma cells. *Oncogene* 16:9–20
  40. Valles AM, Boyer B, Badet J, Tucker GC, Barrisault D, Thiery JP (1990) Acidic fibroblast growth factor is a modulator of epithelial plasticity in a rat bladder carcinoma cell line. *Proc Natl Acad Sci U S A* 87:1124–1128
  41. Morali OG, Delmas V, Moore R, Jeanney C, Thiery JP, Larue L (2001) IGF-II induces rapid beta-catenin relocation to the nucleus during epithelium to mesenchyme transition. *Oncogene* 20:4942–4950
  42. Yamashita S, Miyagi C, Fukada T, Kagara N, Che YS, Hirano T (2004) Zinc transporter LIV1 controls epithelial-mesenchymal transition in zebrafish gastrula organizer. *Nature* 429:298–302
  43. Sasaki H, Sato Y, Kondo S, Fukai I, Kiriya M, Yamakawa Y, Fujii Y (2002) Expression of the periostin mRNA level in neuroblastoma. *J Pediatr Surg* 37:1293–1297
  44. Kudo Y, Ogawa I, Kitajima S, Kitagawa M, Kawai H, Gaffney PM, Miyauchi M, Takata T (2006) Periostin promotes invasion and anchorage-independent growth in the metastatic process of head and neck cancer. *Cancer Res* 66:6928–6935
  45. Chang Y, Lee TC, Li JC, Lai TL, Chua HH, Chen CL, Doong SL, Chou CK, Sheen TS, Tsai CH (2005) Differential expression of osteoblast-specific factor 2 and polymeric immunoglobulin receptor genes in nasopharyngeal carcinoma. *Head Neck* 27:873–882
  46. Puppini C, Fabbro D, Dima M, Di Loreto C, Puxeddu E, Filetti S, Russo D, Damante G (2008) High periostin expression correlates with aggressiveness in papillary thyroid carcinomas. *J Endocrinol* 197:401–408
  47. Siriwardena BS, Kudo Y, Ogawa I, Kitagawa M, Kitajima S, Hatano H, Tilakaratne WM, Miyauchi M, Takata T (2006) Periostin is frequently overexpressed and enhances invasion and angiogenesis in oral cancer. *Br J Cancer* 95:1396–1403
  48. Shao R, Bao S, Bai X, Blanchette C, Anderson RM, Dang T, Gishizky ML, Marks JR, Wang XF (2004) Acquired expression of periostin by human breast cancers promotes tumor angiogenesis through up-regulation of vascular endothelial growth factor receptor 2 expression. *Mol Cell Biol* 24:3992–4003
  49. Sasaki H, Dai M, Auclair D, Kaji M, Fukai I, Kiriya M, Yamakawa Y, Fujii Y, Chen LB (2001) Serum level of the periostin, a homologue of an insect cell adhesion molecule, in thymoma patients. *Cancer Lett* 172:37–42
  50. Sasaki H, Yu CY, Dai M, Tam C, Loda M, Auclair D, Chen LB, Elias A (2003) Elevated serum periostin levels in patients with bone metastases from breast but not lung cancer. *Breast Cancer Res Treat* 77:245–252
  51. Sasaki H, Dai M, Auclair D, Fukai I, Kiriya M, Yamakawa Y, Fujii Y, Chen LB (2001) Serum level of the periostin, a homologue of an insect cell adhesion molecule, as a prognostic marker in non-small cell lung carcinomas. *Cancer* 92:843–848
  52. Soltermann A, Ossola R, Kilgus-Hawelski S, von Eckardstein A, Suter T, Aebersold R, Moch H (2008) N-glycoprotein profiling of lung adenocarcinoma pleural effusions by shotgun proteomics. *Cancer* 114:124–133
  53. Sasaki H, Lo KM, Chen LB, Auclair D, Nakashima Y, Moriyama S, Fukai I, Tam C, Loda M, Fujii Y (2001) Expression of periostin, homologous with an insect cell adhesion molecule, as a prognostic marker in non-small cell lung cancers. *Jpn J Cancer Res* 92:869–873
  54. Pirinen R, Leinonen T, Bohm J, Johansson R, Ropponen K, Kumpulainen E, Kosma VM (2005) Versican in non-small cell lung cancer: relation to hyaluronan, clinicopathologic factors, and prognosis. *Hum Pathol* 36:44–50
  55. Tsunoda T, Furusato B, Takashima Y, Ravulapalli S, Dobi A, Srivastava S, McLeod DG, Sesterhenn IA, Ornstein DK, Shirasawa S (2009) The increased expression of periostin during early stages of prostate cancer and advanced stages of cancer stroma. *Prostate* 69:1398–1403
  56. Castronovo V, Waltregny D, Kischel P, Roesli C, Elia G, Rybak JN, Neri D (2006) A chemical proteomics approach for the identification of accessible antigens expressed in human kidney cancer. *Mol Cell Proteomics* 5:2083–2091
  57. Morra L, Rechsteiner M, Casagrande S, Duc Luu V, Santimaria R, Diener PA, Sulser T, Kristiansen G, Schraml P, Moch H, Soltermann A (2011) Relevance of periostin splice variants in renal cell carcinoma. *Am J Pathol* 179:1513–1521
  58. Li JS, Sun GW, Wei XY, Tang WH (2007) Expression of periostin and its clinicopathological relevance in gastric cancer. *World J Gastroenterol* 13:5261–5266
  59. Ben QW, Jin XL, Liu J, Cai X, Yuan F, Yuan YZ (2011) Periostin, a matrix specific protein, is associated with proliferation and invasion of pancreatic cancer. *Oncol Rep* 25:709–716
  60. Tilman G, Mattiussi M, Brasseur F, van Baren N, Decottignies A (2007) Human periostin gene expression in normal tissues, tumors and melanoma: evidences for periostin production by both stromal and melanoma cells. *Mol Cancer* 6:80
  61. Hoersch S, Andrade-Navarro MA (2010) Periostin shows increased evolutionary plasticity in its alternatively spliced region. *BMC Evol Biol* 10:30
  62. Kim CJ, Isono T, Tambe Y, Chano T, Okabe H, Okada Y, Inoue H (2008) Role of alternative splicing of periostin in human bladder carcinogenesis. *Int J Oncol* 32:161–169

63. Wang ET, Sandberg R, Luo S, Khrebtkova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP, Burge CB (2008) Alternative isoform regulation in human tissue transcriptomes. *Nature* 456:470–476
64. Nilsen TW, Graveley BR (2010) Expansion of the eukaryotic proteome by alternative splicing. *Nature* 463:457–463
65. Warzecha CC, Jiang P, Amirikian K, Dittmar KA, Lu H, Shen S, Guo W, Xing Y, Carstens RP (2010) An ESRP-regulated splicing programme is abrogated during the epithelial-mesenchymal transition. *EMBO J* 29:3286–3300
66. Neri D, Bicknell R (2005) Tumour vascular targeting. *Nat Rev Cancer* 5:436–446
67. Bischoff J (1995) Approaches to studying cell adhesion molecules in angiogenesis. *Trends Cell Biol* 5:69–74
68. Kudo Y, Siriwardena BS, Hatano H, Ogawa I, Takata T (2007) Periostin: novel diagnostic and therapeutic target for cancer. *Histol Histopathol* 22:1167–1174
69. Hehlhans S, Haase M, Cordes N (2007) Signalling via integrins: implications for cell survival and anticancer strategies. *Biochim Biophys Acta* 1775:163–180